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Liver Fatty Acid-Binding Protein Is a New Prognostic Factor for Hepatic Resection of Colorectal Cancer Metastases

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Background and Objectives: Liver fatty acid-binding protein (L-FABP) is reported as a biological marker for enterocytic differentiation. We evaluated the prognostic value of L-FABP expression for patients undergoing hepatic resection of colorectal cancer metastases.

Methods: The study group comprised 68 patients who underwent hepatic resection for colorectal cancer metastases between 1982 and 1996 at Niigata University Medical Hospital, Niigata, Japan. L-FABP expression was immunohistochemically studied in metastatic liver tumors and their primary colorectal cancers. The relationship between L-FABP expression and patient prognoses was statistically analyzed.

Results: L-FABP was positively stained in 56% (38/68) of liver metastases from colorectal cancers and in 56% (38/68) of their primary tumors. Of 68 cases, 54 (79%) showed similar immunohistochemical findings between primary and metastatic tumors. Patients with L-FABP-positive liver metastases showed better prognosis than patients with L-FABP-negative metastases ($P = 0.046$). L-FABP expression in primary colorectal cancers more significantly ($P = 0.009$) affected long-term survival after hepatic surgery. Multivariate analysis revealed that the prognostic effect of L-FABP expression in primary colorectal cancers was exerted independently and that its impact was larger than conventional pathological prognosticators.

Conclusions: L-FABP expression is suitable for use as a new presurgical prognostic factor for patients undergoing hepatic surgery for colorectal cancer metastases.

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KEY WORDS: immunohistochemistry; cell differentiation; multivariate analysis

INTRODUCTION

Hepatic resection is the standard and only curative treatment for colorectal cancer metastases to the liver and results in 5-year survival rates of 25–35% [1,2]. Unfortunately, the procedure also has occasional adverse effects. Proper selection of patients likely to benefit from the surgery is important for more effective treatment; however, criteria for such selection remain subjective at present.

Many studies have attempted to determine predictive factors for prognosis of patients undergoing hepatic resection for colorectal cancer metastases [1–6]. These reports have indicated that surgical margin, number of metastases, and stage of primary tumor are the main predicting factors. However, there remains controversy as to which technical or pathological factors are useful predictors of long-term survival.

Liver fatty acid-binding protein (L-FABP) is specifically expressed in hepatocytes and enterocytes [7]. This 14-kDa protein is a sensitive marker of enterocytic dif-

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ferentiation [8]. We hypothesized that L-FABP expression in colorectal carcinoma would be a useful indicator for hepatic surgery. This study evaluates the prognostic significance of L-FABP expression following hepatic resection of colorectal cancer metastases.

MATERIALS AND METHODS

Patients

We retrospectively reviewed 75 consecutive patients who underwent curative-intent hepatic resection for colorectal cancer metastases at Niigata University School of Medicine, Niigata, Japan, between 1982 and 1996. One patient who died of hepatic failure within 30 days of surgery was excluded from consideration. Six patients whose resected specimens were lost were also excluded. The remaining 68 patients included 40 men and 28 women ranging in age from 32 to 78 years (average, 58).

Using an immunohistochemical technique, we examined all 68 patients for L-FABP expression in liver metastases and their primary colorectal cancers.

Immunohistochemical Analysis

A polyclonal rabbit anti-L-FABP serum cultured against rat L-FABP (a generous gift of Dr. T. Ono, Department of Biochemistry, Niigata University School of Medicine) was used for the immunohistochemical staining. Western blot analysis was used to ensure the specificity of the antiserum [9].

Formalin-fixed, paraffin-embedded sections were investigated using a Histofine rabbit streptavidin-biotin-perioxidase kit (SAB-PO®, Nichirei Corp., Tokyo, Japan) according to the manufacturer's instructions. Negative controls were prepared by substituting preimmunized rabbit serum for the primary antiserum.

Analysis of all sections was blinded to both treatment outcomes and clinicopathologic features. On the basis of the percentage of positively staining cancer cells, tumors were classified as either L-FABP negative (positive cells <10%) or positive (≧10%).

Statistical Analysis

The StatView® (Abacus Concepts, Inc., California) statistical package was used for the statistical computation. Survival curves were calculated according to the Kaplan-Meier method and compared using the log-rank test. The impact of prognostic factors was studied by both univariate analysis and stepwise multivariate analysis using the Cox proportional hazards model. $P < 0.05$ was considered significant.

RESULTS

Immunohistochemical Staining for L-FABP

Figure 1 shows L-FABP immunohistochemistry in primary colorectal carcinoma and hepatic metastases. L-FABP-positive cells of tumors showed a diffuse and cytoplasmic staining pattern similar to that observed in immunoreactive normal enterocytes and hepatocytes. Distribution of the L-FABP-positive cells varied with their type, i.e., patchy, mosaic, or diffuse.

Of 68 patients, 30 (44%) were negative and 38 (56%) were positive for L-FABP immunostaining. In 54 cases (79%), L-FABP immunoreactivity was unchanged between the primary tumor and metastatic liver tumor; however, 14 cases (21%) showed immunochemical conversion (Table I). In cases of multiple liver metastases, the tumors shared similar immunohistochemical findings.

Survival After Hepatic Resection

The overall 5-year survival rate of the 68 patients in this series was 33%. The 5-year survival of patients with L-FABP-negative liver metastases was 23%, and that of patients with L-FABP-positive metastases was 40% (Fig. 2A). This prognostic difference was statistically significant ($P = 0.046$).

With regard to the immunochemical evaluation of their primary colorectal tumors, the overall survival of patients with L-FABP-positive primary colorectal tumors was significantly better than that of patients with L-FABP-negative primary tumors (5-year survival: 43% vs. 9%, $P = 0.009$) (Fig. 2B).

We also examined clinical or pathological factors influencing the prognosis of patients following hepatic resection (Table II). Log-rank tests showed that of the 12 clinicopathological factors, only marginal clearance ($P = 0.046$) and size of the largest hepatic metastasis ($P = 0.018$) had prognostic significance. Although number of hepatic metastases ($P = 0.053$) and location on the liver ($P = 0.092$) showed a tendency to affect survival, the other 8 factors, including Dukes stage and DNA ploidy of the primary tumors, had no prognostic impact.

Univariate and Multivariate Analyses of Prognostic Determinants

To better understand the prognostic implications of L-FABP expression, we used Cox regression to compare the impacts of the 6 candidates for prognostic determinants revealed by the log-rank tests ($P < 0.1$). Univariate analysis showed that L-FABP expression in primary tumors ($P = 0.011$), L-FABP expression in liver metastases ($P = 0.049$), and number of hepatic metastases ($P = 0.022$) were statistically significant factors. Since a relationship was observed between L-FABP expressions of primary tumors and liver metastases, we included L-FABP expression in primary tumors as a factor in our multivariate analysis. Multivariate analysis confirmed that the other 4 factors (marginal clearance, size of the largest metastasis, number of metastases, and location on the liver) were not significantly related to L-FABP expression. It also showed that L-FABP expression in primary tumors was the most prognostic factor, with a relative risk of 2.33 ($P = 0.016$) (Table III).

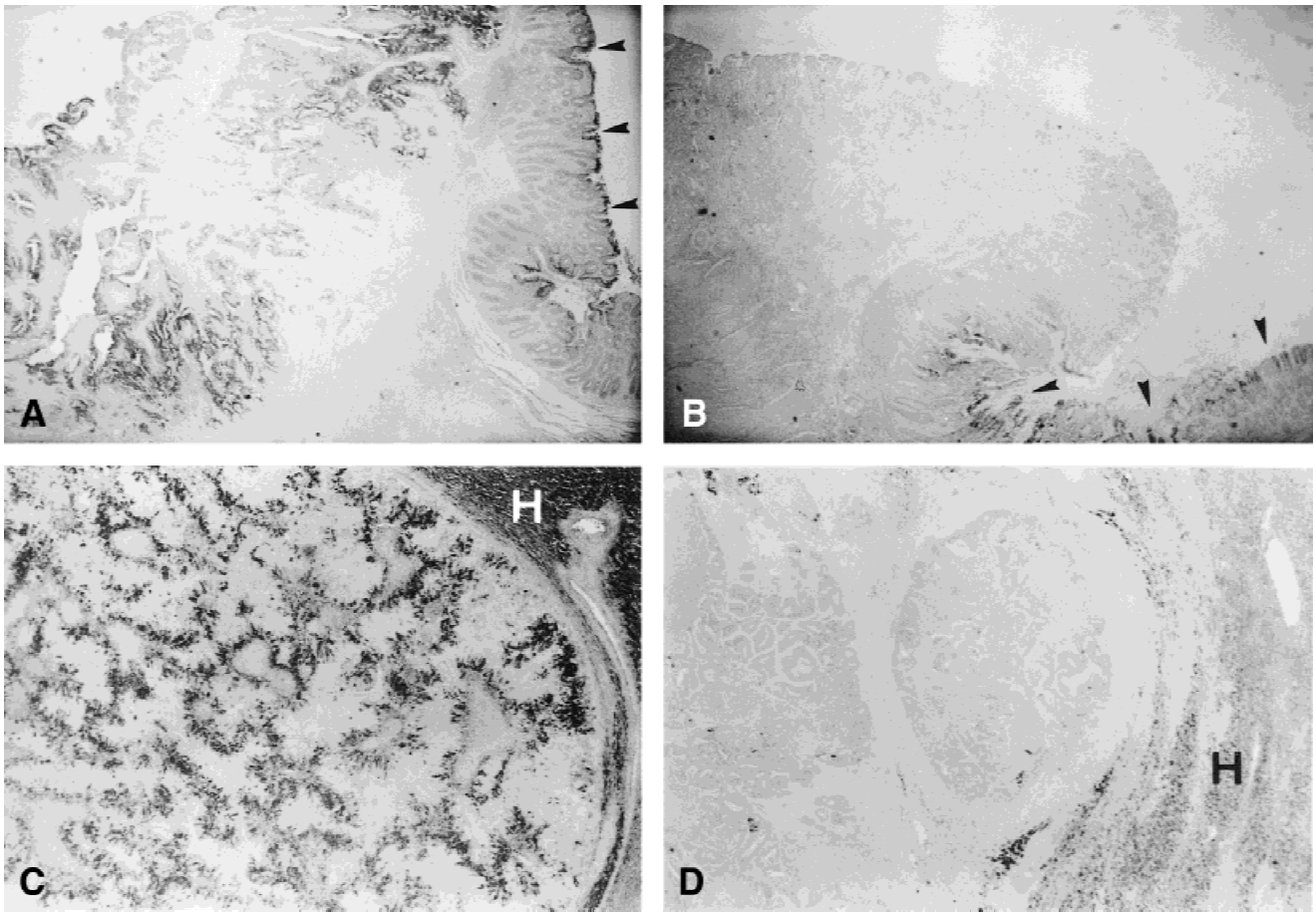

Fig. 1. Immunostaining of L-FABP-positive **(A)** and L-FABP-negative **(B)** primary colorectal cancers, and of L-FABP-positive **(C)** and L-FABP-negative **(D)** liver metastases. Normal enterocytes (arrowheads) located in the surface epithelium and the upper portion of the crypt are immunoreactive. Hepatocytes (H) are diffusely stained by L-FABP immunohistochemistry. ×8.

TABLE I. Relationship Between L-FABP Immunostaining of Primary Tumors and Liver Metastases

L-FABP expression			No. of patients (n = 68)
Primary tumors	⟶	Liver metastases	
Negative	⟶	Negative	23
Negative	⟶	Positive	7
Positive	⟶	Negative	7
Positive	⟶	Positive	31

DISCUSSION

In the present study, we demonstrate a new biological marker for the evaluation of patient prognosis following hepatic resection of colorectal cancer metastases. Patients bearing L-FABP-positive colorectal cancer showed better prognosis after hepatic resection than those bearing L-FABP-negative cancer. The prognostic difference was generated not only by L-FABP expression in hepatic metastases but also by such expression in the primary colorectal cancer. These results indicate that the properties of cancer cells are inherited from primary tumors to metastatic tumors. The prognostic variable may be already present in the primary tumor, and the metastasis follows the prognostic indicators of the primary tumor.

To recognize a factor that affects patient survival as a prognostic determinant, the factor should be characterized by a sufficiently large impact and should be completely independent of other factors affecting the prognosis. Statistical analyses in the present study revealed that the prognostic effect generated by L-FABP expression is significant and comparable with conventional pathological factors, such as surgical margin and size, number, and location of hepatic metastases. Furthermore, multivariate analysis confirmed that the prognostic effect of L-FABP expression was independent of and larger than the impacts of the other 4 clinicopathological factors.

Many studies have reported prognostic factors for patients undergoing hepatic resection for colorectal metastases [1–6]. However, controversy remains in regard to which preoperative features are useful predictors of long-term survival. For this reason, we also analyzed the prognostic effects of possible predictors reported by other groups, including Dukes stage [2,6], preoperative car-

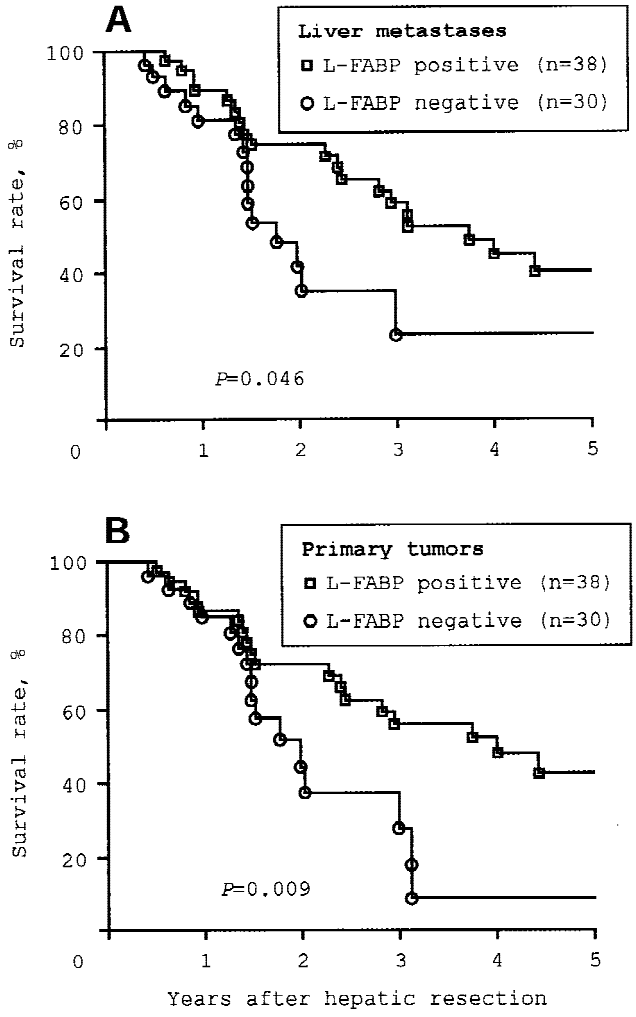

Fig. 2. Relationship between L-FABP expression and survival after hepatic resection of colorectal cancer metastases. **(A)** Survival in patients with L-FABP-positive and L-FABP-negative liver metastases. The difference is statistically significant ($P = 0.046$). **(B)** Survival in patients with L-FABP-positive and L-FABP-negative primary colorectal cancers. The difference is statistically significant ($P = 0.009$).

cinoembryonic antigen (CEA) level [3,5], and DNA ploidy [5,10]. In our series, none of these factors significantly affected the probability of survival after hepatic resection of colorectal metastases.

Our statistical analysis showed that L-FABP expression in primary tumors had a larger prognostic impact than it did in metastatic tumors (univariate relative risk, 2.44 vs. 2.02). This finding indicates at least a clinical benefit of L-FABP immunohistochemistry. To identify a good candidate for hepatic resection of colorectal cancer metastases, information about L-FABP expression of the primary colorectal cancer is preoperatively available from the biopsy specimen in synchronous cases and from formalin-fixed samples in metachronous cases. This suggests, in combination with the big and independent prog-

TABLE II. Survival After Hepatic Resection According to Clinicopathological Factors and L-FABP Expression (Log-Rank Test)

Factors	No. of patients (n = 68)	5-Year survival rate (%)	*P*
Age (years)			
<60	32	36	0.167
≧60	36	28	
Sex			
Male	40	39	0.283
Female	28	28	
Histological type			
Well differentiated	40	45	0.137
Moderately differentiated	24	21	
Unknown	4		
Stage at first surgery			
Dukes A, B	16	37	0.467
Dukes C	20	30	
Dukes D	31	37	
Unknown	1		
DNA ploidy of primary tumors			
Diploid	25	32	0.788
Aneuploid	36	27	
Unknown	7		
L-FABP expression in primary tumors			
Positive	38	43	0.009
Negative	30	9	
Time interval from primary tumor[a]			
Synchronous	29	34	0.646
Metachronous	39	33	
Preoperative CEA level (ng/ml)			
<5	13	64	0.191
≧5	52	25	
Unknown	3		
Marginal clearance (mm)			
<5	31	20	0.046
≧5	33	45	
Unknown	4		
Size of the largest metastasis (cm)			
<5	46	42	0.018
≧5	21	12	
Unknown	1		
No. of metastases			
1–4	64	36	0.053
≧5	4	0	
Type of hepatic resection[b]			
Major	45	36	0.763
Minor	23	27	
Location on the liver			
Unilateral	48	38	0.092
Bilateral	20	23	
L-FABP expression in liver metastases			
Positive	38	40	0.046
Negative	30	23	

[a]Synchronous metastases occurred within 3 months from primary tumor.
[b]Major resections were defined as at least segmentectomies.

TABLE III. Univariate and Multivariate Analyses of Prognostic Determinants (Cox Proportional Hazards Model)

	Univariate			Multivariate		
Factors	RR[a]	χ^2	*P*	RR[a]	χ^2	*P*
Marginal clearance	1.98	3.82	0.051	—	3.97	0.046
Size of the largest metastasis	2.72	5.29	0.063	—	4.72	0.030
No. of metastases	2.29	3.45	0.022	—	3.35	0.067
Location on the liver	1.79	2.77	0.096	—	2.62	0.105
L-FABP expression in primary tumors	2.44	6.43	0.011	2.33	5.84	0.016
L-FABP expression in liver metastases[b]	2.02	3.86	0.049			

[a]RR, relative risk.
[b]This factor was excluded from the multivariate model.

nostic impact, that L-FABP expression is a promising marker for patient selection in hepatic resection.

This independent predictability of L-FABP may be exemplified by cases we followed in the present study. A 47-year-old man underwent surgery three times for developing multiple metachronous liver metastases after resection of sigmoid colon cancer and synchronous liver metastasis. The patient, whose L-FABP staining was positive in both primary and metastatic tumors, has survived >6 years since the first surgery. In contrast, a 59-year-old man, in whom L-FABP staining was negative in both primary and metastatic tumors, underwent surgery for solitary metachronous liver metastasis 4 cm in diameter and died of systemic recurrence 17 months after the hepatic resection.

The reason why L-FABP expression is related to survival is unknown. A study of rat intestinal development [11] has demonstrated that the L-FABP gene is activated at the late gestation period and the number of L-FABP-producing enterocytes increases as the villi elongate and mature. A human enterocyte-like cell line, Caco-2, which is derived from human colonic cancer, commences expression of L-FABP after becoming highly differentiated in its morphology and metabolism [12]. These findings suggest that L-FABP expression may be a sensitive marker for differentiating cancer cells that are morphologically indistinguishable by light microscopy. Histologically undifferentiated cancers in the colon and rectum grow rapidly and aggressively [13,14]. We have observed that L-FABP-negative cancers tend to relapse systemically after hepatic resection (data not shown). Expression of the L-FABP gene could be a sensitive marker reflecting the cellular differentiation and biological virulence of colorectal cancer.

In conclusion, patients with liver metastasis derived from L-FABP-positive colorectal cancer show better prognosis following hepatic surgery than their L-FABP-negative counterparts. Since information about L-FABP immunohistochemistry of colorectal cancer can be obtained before hepatic surgery, L-FABP immunostaining should serve as a useful presurgical prognostic indicator.

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